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Chemical Investigations and the Antimicrobial Activity of *Ocimum Hadiensis (Forssk)* Plant Grown Wild in Egypt

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Abstract

In the framework of our studies for the survey and evaluation of Egyptian wild plants, this study includes the examination and evaluation of the *Ocimum hadiensis* which grows wild in the Egyptian desert. The collection of the plant material was carried out from the plants growing in Egyptian eastern desert, about 1200 km from Cairo. The chemical constituents of the volatile oil of *O. Hadiensis* leaves were analyzed by GC and GC-MS. Twenty one compounds representing 97.02% of the essential oil was identified. The main compounds of *O. hadiensis* are methyl eugenol (78.24%), caryophyllene oxide (3.64 %), alpha-trans-bergamotene (3.2 %), β–caryophyllene (1.75 %), piperitone (1.66 %), eugenol (1.65 %) and linalool (1.1 %). The antimicrobial activity was tested using Gram negative bacteria, Gram positeve bacteria, yeast and fungi. Water extract of *O. hadiensis* plant under investigation was effective on *Proteus. vulgaris* (25.00), *Salmonella typhi* (13.00), and *Candida albicans* (inhibition zone 15.00 mm in diameter), while it had moderate effect with fungi *Aspergillus niger*. Low effect was observed only as a result of the use of 80 % alcoholic extract on strains of bacteria (G -) (*Escherichia coli* and *Proteus. vulgaris*), bacteria (G +) : *Bacillus subtilis, Lactobacillus breveis* ,Yeast : *Candida* albicans and strains of fungi: *A. Niger*.

1. Introduction

There has been an increment in the demand for wild medicinal plants to be used in traditional medicine as well as contemporary and alternative medicine in developing and developed countries [1]. Not only for cheap herbal remedies, but also, it is safer than using chemically manufactured drugs with little or no side effects [2]. Herbal medicines, because of their decentralized nature, are generally readily available. Medicinal and aromatic plants are now an important pillar in the pharmaceutical, food and perfumery industries. Medicinal plants are important for pharmacological research, drug development, and raw materials for drug synthesis or as models for pharmaceutical active compounds [3]. The sustainable development of traditional medicine in developing countries requires a serious investment in the maintenance of medicinal plants and traditional medicine to provide the better health care. The geographical nature of Egypt is characterized by the presence of five natural geographical areas including Western Desert, Eastern Desert, Nile Valley, Nile Delta and the Sinai Peninsula, making it a fertile environment for the growth of different types of medicinal and aromatic plants, whether wild or cultivated [4]. Since most of the Egyptian lands are desert, it was therefore necessary to explore the growth areas of these plants in the Egyptian deserts and follow their places of growth, which are mostly related to the rainy season. In this context and within the framework of a program focuses on the study of aromatic and wild plants, such as *Ocimum hadiensis (Forssk)* [or *Plectranthus hadiensis (Forssk)* var. hadiensis], common name-hairy Spurflower (Lamiaceae). It is an attractive perennial shrub with decorative foliage. The species name *hadiensis* is derived from the Arabic name from, Hadiyada in the mountains of Yemen where it was collected first [5]. According to the literature *P. hadiensis* is mainly used for diseases related to the digestive system such as dysentery, vomiting, diarrhea and acute and chronic liver congestion [6]. Phytochemical studies of Indian *P. hadiensis* revealed that it is rich in essential oil. The volatile oil is obtained only from very few species of *Plectranthus*. Their oil composition has been reviewed and reported [7].

Twenty five compounds representing 99.3% of the *P. hadiensis seeds* essential oil from India were identified. The main compound of *P. hadiensis* seed oil is piperitone oxide (33.33%). The antibacterial activity of *P. hadiensis* seed essential oil is tested against two Gram-positive and two Gram-negative bacteria, by using the method of inhibition zone. The volatile oils of *P. hadiensis* inhibit the organisms and show the zone of inhibition in the range of 20-35mm. Essential oil of *P. hadiensis* can act as an antibacterial agent [8]. Several investigators reported that, the secondary compounds show the therapeutic activity against various diseases in human categories, thus can be used as traditional medicinal plants to treat some diseases [9,10]. The aim of this work is to study the essential oil compositions, phytochemical screening and antimicrobial activities of *Ocimum hadiensis*) as a natural product under Egyptian conditions.

2. Materials and methods

2.1. Plant material

The aerial parts of *O. Hardy* was collected from Gebel Elba region approximately 1200 km south of Cairo in March 2013 (During flowering stage). Identification of the species was achieved by Prof. Dr. Loutfy Boulos [11-12]. Voucher specimens are in the herbarium of NRC, Cairo, Egypt.

2.2. Essential oil isolation

Five hundred grams from dried herb were subjected to hydro-distillation for 3 hrs using a Clevenger-type apparatus [13]. The essential oil content was calculated as a relative percentage (v/w). The samples of the essential oils were dehydrated over anhydrous sodium sulphate and stored in refrigerator until analyzed.

2.3 Gas chromatography (GC)

GC analyses were performed using a Shimadzu GC- 9A gas chromatograph equipped with a DB5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was held at 40°C for 5 min and then programmed until 250°C at a rate of 4°C/min. Injector and detector (FID) temperature were 260°C; helium was used as carrier gas with a linear velocity of 32 cm/s.

2.4. Gas chromatography-mass spectrometry (GC-MS)

Gas-chromatograph apparatus was used. A capillary DB5 (me- thyl‑silicone containing 5% phenyl groups) column (30 m × 0.25 mm i.d.) was used. Temperature program: 2 min at 60 °C, 60–100 °C (2 °C/ min) and 100–250 °C (5 °C/min). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. Injection volume: 1.0 μL at a 1:50 split. A mass spectrometer (EI-MS 70 eV) was used by using a spectral range of *m*/*z* 40–350.

2.5. Qualitative and quantitative analyses of essential oil

Identifications were made by library searches (Adams, 1995) [14] combining MS and retention data of authentic compounds by comparison of their GC retention indices (RI) with those of the literature or with those of standards available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C8–C22) [15] under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley5 Libraries or with mass spectra from literature. Component relative concentrations were calculated based on GC peak areas without using correction factors.

2.6. Preparation of the crude extracts

2.6.1. Alcoholic extract

The 80% ethanolic extracts were prepared following the process described [16]; One hundred gm of the aerial parts of the plant were collected, dried in the oven at 40 °C and reduced to powder. They were macerated with the 80% ethanol and allowed to stand for 72 hrs and then filtered. The filtrate was then evaporated under reduced pressure and dried using a rotary evaporator at 50°C. Dried extracts were stored in labelled sterile screw capped bottles at 5°C in the refrigerator, until when required for use.

2.6.2.Water extract

One hundred gm of the dried powder of the plant were macerated in water at room temperature for 24 hrs. The macerates were filtered and evaporated under vacuum till dryness. The residues were kept for testing and antimicrobial activity.

2.7. Phytochemical screening:
The powdered air-dried aerial parts of plant under study was screened for carbohydrates and / or glycosides; sterols and / or triterpenes, flavonoids, tannins, saponins, coumarins and alkaloids, applying chemical tests [17].

2.8. Biological Activity:
2.8.1 Plant extraction
The dried aerial parts of the tested herb were extracted by 80% alcohol. The extract was evaporated under vacuum and the residue was dissolved in alcohol to give concentration of100 µg/ml.

2.8.2. Microbiological techniques
Microbial strains: The antimicrobial activity of alcoholic and water extracts were tested against some bacterial strains (Gram negative bacteria: *Escherichia coli, Proteus vulgaris, Salmonella typhi.* (Gram positive bacteria: *Bacillus subtilis, Pseudomonas fluroscens, Lactobacillus breveis, Staphylococcus aureus* and *chromobacter sp*), one fungal strain (*Aspergillus niger*) and one yeast strain (*Candida albicans*). Tested organisms were obtained from the Faculty of Agriculture, Cairo University.

2.9. Agar diffusion method
The method of agar diffusion assay was carried out [18]. Nutrient agar was used for the cultivation of bacteria and yeast; while Czapek-Dox's medium (Dox 1910) was used for cultivation of fungal species. In this method, pre- sterilized Whatman no.1 filter paper discs (5 mm in diameter) (Whatman International Ltd., Maidstone, England) were impregnated with 100 µl of the extract (100 µg/ml) and was allowed to dry (to get rid of the alcohol) and was then applied on the surface of agar plates freshly seeded with standard inoculate of young cultures, 24-hrs-old bacteria and yeast, and 7-days-old fungi. The plates of test organisms were then incubated at 27ºC for 24 hrs for bacteria & yeast and for 48 hrs for fungi. At the end of the incubation period, the inhibition zones were measured (results are the average of triplicate measurements).

2.10. Statistical analysis of data
All values of the antimicrobial activity were expressed as the mean of inhibition zones (mm) with three replicates for each treatment. Data were subjected to a paired-sample *t*-test using SPSS (ver. 9.0). $P<0.05$ was regarded as significant.

3. Results and discussion
It was noted that most Egyptian wild plants grow in the beginning of the rainfall in Egyptian desert areas (starting in October). So, survey and collection of most wild medicinal plants of various Egyptian eastern desert areas were collected during the period from October through May of each year. Therefore, the plant materials of *O. hadiensis* were collected from wild shrub populations growing in sandy soils on Gebel Elba region (Wadi Yahmib) during November and December 2016.

3.1. Phytochemical screening of the dried herb of O. hadiensis
Data in Table (1) recorded the preliminary phytochemical screening of the dry *O. hadiensis* herb in the flowering stage. Phytochemical investigation of the alcoholic extracts reported that it is rich in essential oils (Natural components, characterized by, a strong aromatic odor). Main uses of essential oils: medical treatment, therapeutic massage, skin cosmetics, hair care, mineral water treatment, perfumes, cosmetics and gastronomy. There are a highly concentrated plant extract for all adsorption methods

Table 1.Phytochemical screening of *Ocimum hadiensis*

Chemical group	Phytochemical screening
Essential oils	High value (++)
Carbohydrates and / or glycos	Low value (+)
Sterols& terpenes	Low value (+)
Flavonoids	Low value (+)
Tannins	Moderate Value (±)
Alkaloids	Absent (-)
Saponins	Absent (-)
Coumarins	Low value (+)
Anthraquinones	Absent(-)

In additions the dried herb of *Ocimum hadiensis* contains low amounts of carbohydrates sterols, flavonoids and coumarins. Tannins were found in moderate amount, while the absence of alkaloids, saponins and anthraquinones was observed.

3.2 Essential oil constituents of O. hadiensis

The volatile oil isolated by water distillation from herb of *O. handiness* yielded about 0.43% (v/w) based on a dry weight. Twenty one compounds representing 97.02% were identified (Table 2).The main compounds are methyl eugenol (78.24%), caryophyllene oxide (3.64%), alpha-trans-bergamotene (3.20%), β−caryophyllene (1.75%), piperitone (1.66%), eugenol(1.65%) and linalool (1.10%). The main compound of methyl eugenol is a yellowish, oily, liquid naturally occurring with a clove-like aroma. Methyl eugenol is used as a flavor and fragrance agent. It is a constituent of a large number of essential oils obtained from many plants, e.g., *Daucus carota, Artemisia dracunculus* and *Cinnamomum oliveri* leaves (90–95%) [19]. Methyl eugenol is a powerful inhibitor of the enzyme acetylcholinesterase [20], responsible for the decomposition of the neurotransmitter acetylcholine, which can eventually lead to paralysis in insects.

Table 2. Chemical constituents of essential oil extracted from *O. hadiensis* (Forssk.)

Peak No	Compound	KI	%	Group	Formula
1	4-pentenal	697	0.21	VC	C5H8O
2	3-Ethoxy-1-propanol	761	0.31	VC	C5H12O2
3	α−pinene	939	0.26	MHC	C10H16
4	Sabinene	976	0.11	MHC	C10H16
5	Octanol 2	997	0.22	VC	C8H18O
6	α−terpinene	1018	0.13	MHC	C10H16
7	Limonene	1031	0.41	MHC	C10H16
8	Linalool	1098	1.10	OMC	C10H18O
9	α−terpineol	1189	0.42	OMC	C10H18O
10	Piperitone	1252	1.66	OMC	C10H16O
11	Eugenol	1356	1.65	OMC	C10H12O2
12	Copaene	1376	0.80	OMC	C15H24
13	Methyl eugenol	1401	78.24	VC	C11H14O2
14	β−caryophyllene	1418	1.75	SHC	C15H24
15	alpha-trans-bergamotene	1436	3.20	SHC	C15H24
16	α− patchoulene	1456	1.10	SHC	C15H24
17	β−farnesene	1458	0.21	SHC	C15H24
18	α -muurolene	1477	0.32	SHC	C15H24
19	δ-Cadinene	1530	1.09	SHC	C15H24
20	Caryophyllene oxide	1581	3.64	OSC	C15H24O
21	Farnesol	1722	0.19	OSC	C15H26O
Total (All)			97.02		

Essential oil of *O. hadiensis* (Forssk.) from Egypt are classified into five groups (Table 3) which are monoterpene hydrocarbons compounds (MHC), oxygenated monoterpene compounds (OMC), sesquterpenes hydrocarbons compounds (SHC), oxygenated sesquterpenes compounds (OSC) and various compounds group (VC). Data in the Table 3 recorded that the (VC) group gave the highest concentrations, followed by (SHC), (OMC), (SOC) and (MHC) group.Methyl eugenol is the major constituents of the (VC) group of *O. handiness* essential oil. Other minor compounds in this group were detected as, 3-ethoxy-1-propanol, 4-pentenal and octanol 2. The main constituents of (SHC) were β−caryophyllene, α−patchoulene, alpha-trans-bergamotene, beta-farnesene, α−muurolene and delta – cadinene. (OMC) group consists of five compounds. Linalool, α−terpineol, eugenol, copaene and, piperitone. (MHC), group consists of four compounds, α−pinene, sabinene, α−terpinene and limonene. Concerning (OSC) group, it contains only two compounds, caryophyllene oxide and farnesol. From the above data, it was noted that there are many differences in the composition of *O. handiness*

oil produced under Egyptian condition in comparison with the oil produced under the other conditions [7], especially, the content of the main constituents. In Egypt the main components of *O. handiness* were methyl eugenol (78.24 %) and caryophyllene oxide (3.64%), while, under the Indian conditions, the two major constituents of seed oil were piperitone oxide (33.33%) and copaene (8.82%). Similar results were reported by Galambosi *et. al.*[21] who suggested some chemo-types of some medicinal and aromatic plants based on the dissimilar biosynthetic pathways which reflected the chemical content of the volatile oils formed under different region conditions. So, terpenoids as natural products varies by regions with different conditions.

Table 3. Percentage of different chemical groups in *O. hadiensis* oil.

Compound	%
Monoterpenes hydrocarbon (MHC)	0.91
Oxygenated monoterpenes (OMC)	5.63
Sesquterpenes hydrocarbons (SHC)	7.67
Oxygenated sesquterpenes (OSC)	3.83
Various compounds (VC)	78.98
Total	97.02

3.3 Antimicrobial activity

Antimicrobial activity has been tested using (gram negative bacteria): *E. coli, P. vulgaris, P. fluroscens S. typhi*, (gram positive bacteria): *B. subtilis-NRRL–B543 , C. sp, L. breveis, S. aureus*,(yeast) : *C. albicans* and (fungi): *A. niger*. Data in Table 4 showed that, the investigated water extract of the plants was effective against Bacteria (G -):*Proteus vulgaris* (inhibition zone 25.00 mm in diameter) and *S. typhi* (inhibition zone 13.00 mm in diameter), against yeast *C. albicans* (inhibition zone 15.00 mm in diameter) while it has moderate effect with fungi (*Aspergillus niger).* On the other hand no effect was observed due to the use of water extract of plant on the strain of bacteria (G -): *E. coli, and P. fluroscens.* Also no effect for water extract of *O. hadiensis* on the (bacteria G -) *Bacillus subtilis*: *C. sp, L. breveis and S. aureus.* Low effect was observed only as a result of the use of 80 % alcoholic extract on strain of bacteria (G -) (*E. coli and P. vulgaris*), bacteria (G +) : *B. subtilis, L. breveis* ,Yeast : *C. albicans* and strain of fungi: *A. Niger*

Previously, antimicrobial activity of wild plants has been investigated. The literature mentioned many of the biological benefits of wild plants [22-24].

Table 4.Antimicrobial assay of *O. hadiensis* plant growing wild in eastern Egyptian desert.

Strains	Water ext	80 % alcoholic extract	Standard 100µg/disk
Bacteria (G -)	Inhibition zone (mm in diameter) ± SE		
Escherichia coli	0	LW	16.0± 0.6
Proteus vulgaris	25.00 ± 0	LW	
Pseudomonas fluroscens	0	LW	
Salmonella typhi	13.0± 0.1	0	
Bacteria (G +)			
Bacillus subtilis-NRRL–B543)	0	LW	0.5±24.0
Chromobacter sp	0	0	
Lactobacillus breveis	0	LW	
Staphylococcus aureus	0	0	
Yeast			
Candida albicans	15.00± 0	LW	
Fungi			
Aspergillus niger	LW	0	9.0±0.001

0= Not active LW = Low effect

Values were expressed as mean ± SD; (Diameter of inhibition zone including well diameter of 6mm), standard for bacteria: amoxicillin, standard for fungi and yeast: canestin.

Conclusion

Dry herb of *O. hadiensis* has a various chemical groups. It is rich in essential oils. Also herb of *O. hadiensis* contains low amounts of carbohydrates sterols, flavonoids and coumarins. At the same time tannins was found in moderate value, while the absence of alkaloids, saponins and anthraquinones was observed. Twenty one compounds representing 97.02% of the Egyptian *O. hadiensis* essential oil were identified. The main components are methyl eugenol (78.24%), caryophyllene oxide (3.46%), alpha-trans-bergamotene (3.2%), β– caryophyllene (1.75%), piperitone (1.66) and eugenol (1.65%). Other minor compounds were identified. The antimicrobial activity was tested using (G-) bacteria, (G+ bacteria) yeast and fungi. Water extract of *Ocimum hadiensis* under investigation was effective on *P. vulgaris* (25.00), *S. typhi* (13.00), and *C. albicans* (inhibition zone 15.00 mm in diameter), while it was moderate effect with Fungi *A. niger*. Low effect was observed only as a result of the use of 80 % alcoholic extract on strain of Bacteria (G *-) (E. coli and P. vulgaris),* (G +) bacteria *: B. subtilis L. breveis* ,Yeast *: C. albicans* and *strain* of fungi*: A. niger*. Medicinal wild plants have many medicinal uses, making them very useful in the treatment of human diseases. But it must be demonstrated by in vivo experiments.

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